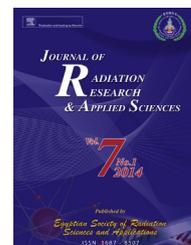


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Effect of kombucha on some trace element levels in different organs of electromagnetic field exposed rats

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ARTICLE INFO

Article history:

Received 25 September 2013

Accepted 11 November 2013

Keywords:

Kombucha

Copper

Zinc

Iron

EMF

Trace element

ABSTRACT

Mobile phones have increased exponentially all over the world. The present study was performed to evaluate the effect of kombucha (KT) on some trace element levels of brain, spleen and intestine in male albino rats exposed to a 950 MHz electromagnetic field (EMF). Four experimental groups labelled as controls, EMF group, KT group and KT + EMF group were formed with six randomly chosen animals in each group. After EMF exposure for eight weeks and the animals were sacrificed by decapitation. Brain, spleen and intestine samples were collected for trace element analysis. The group of animals subjected to electromagnetic waves caused significant increases in iron copper levels and copper/zinc ratio accompanied with a decrease of zinc level in all studied organs. Combined treatment of kombucha with EMF resulted in a successful attenuation of these adverse effects of EMF. From present findings we can state that kombucha as a supplement has an ameliorative signs against the effects of electromagnetic radiation.

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1. Introduction

Trace elements play an important role as activators of enzyme systems or as constituents of organic compounds. For example, zinc and copper play a role in quenching of free radicals through reduction of the peroxidation ratio and breaking the free-radical production chain (Costanzo, De-Guidi, Giuffrida, Sortino, & Condorelli, 1995). On the other hand, iron, copper possesses the ability to generate reactive

radicals, resulting in cellular damage like depletion of enzyme activities, damage to lipid bilayer and DNA (Leonard, Harris, & SHI, 2004; Stohs & Bagchi, 1995). These reactive radical species include a wide variety of oxygen, carbon, sulfur and nitrogen radicals originating not only from superoxide radicals, hydrogen peroxide and lipid peroxidation but also in chelates of amino-acids, peptides and proteins complexes with the toxic metals, which in turn may cause toxicity in different organs of both human and animals (Stohs & Bagchi, 1995). In biological systems, Cu is found mainly in the form of Cu^{2+} , due

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to the oxidation of Cu^{1+} in the presence of oxygen. Cu oxidation is reversible, since Cu^{2+} can accept an electron from strong reductant such as ascorbate and reduced glutathione (Galhard et al., 2004). Cu is an essential cofactor in a number of critical enzymes in metabolism, including superoxide dismutase 1 (Cu/Zn-SOD), cytochrome c oxidase (COX) and ceruloplasmin (CP) (Linder & Hazegh-Azam, 1996). Copper is absorbed in the gut transported to the liver bound to albumin. It enters the blood stream via the plasma protein called ceruloplasmin where its metabolism is controlled and is excreted in bile (Osredkar & Sustar, 2011). However, zinc is absorbed predominantly in the duodenum and jejunum. Pancreatic juice has the highest concentration of zinc and may contain a ligand that promotes absorption of zinc in the intestine. Zinc is exported from the membrane of intestinal epithelial cells into the mesenteric circulation, where it binds to plasma proteins, predominantly albumin circulation. Protein-bound zinc is transported via the portal circulation to the liver (Foote & Delves, 1984). Inadequate zinc absorption causes an immediate decrease in protein turnover and cell growth to preserve zinc body pools. Other elements are co-factors of several antioxidant enzyme systems (Fridovich, 1995). The levels of trace elements can vary considerably depending on age, sex, diet, geographical and climatic conditions, or genetic factors. The concentration of an element may also change by physical or chemical factors (Shen, Li, Zhao, Zhang, & Sun, 2005). An initial rise of trace element concentrations has been reported after the application of radiation (Dede et al., 2003).

Mobile phones devices work in the ultra high frequency range (Ferreira et al., 2006). Their uses have increased exponentially all over the world, leading scientists to become interested in studying their biological effects and the possible consequences on human health. Several studies have shown that mobile phone radiation could cause oxidative stress and damage brain, kidney and heart tissues as well as haematological parameters and nucleic acid (Guney, Ozguner, Oral, Karahan, & Mungan, 2007; Syldona, 2007).

Investigation revealed that kombucha has a wide range of organic and amino acids as well as enzymes that give its extraordinary value. It contains butyric acids that protect the cellular membrane, nucleic acids, RNA and DNA, which transmit information to the cell on how to perform correctly and regenerate. It also contains amino acids which are constituents of proteins, produced antioxidant enzymes and glutathione; a powerful antioxidant, which provides protection from environmental pollutants (Blanc, 1996; Steiger & Steinegger, 1957). Kombucha contains two groups of microorganisms: *Gluconoacetobacter xylinus* ("Xylinum") and yeasts. Xylinum secretes bacterial cellulose through holes in its cell walls. Interestingly, kombucha cultures produce bacterial cellulose more efficiently than cultures of Xylinum alone (Tang, Yang, & Hong, 2012). The bacterial cellulose produced by kombucha cultures has a number of properties that make it potentially useful in medical applications. These properties mean that it acts as a nano-scale functional material with a defined three-dimensional structure and a large number of surface-exposed hydroxyl groups, which allow it to form strong non-covalent bonds with water and also a wide range of ions and organic compounds (Zhu, Li, & You, 2008). It has been

demonstrated that bacterial cellulose efficiently adsorbs numerous toxic heavy metal ions, including Cu^{2+} , Pb^{2+} , Hg^{2+} , and Cd^{2+} . In addition, it adsorbs a range of non-metallic toxins such as histamines, ammonia and NO^{2-} and formaldehyde (Lu & Guan, 2012; Wang & Zhong, 2011; Zhou, Sun, Zhu, & Xu, 2009).

The present work aimed to study the effects of kombucha tea on the levels of iron, copper, zinc and copper/zinc ratio of brain, spleen and intestine tissue in male albino rats exposed to EMF originating from a commercially available mobile phone.

2. Materials and methods

2.1. Animals

Male Wister rats; each weighing 120–140 g, were purchased from the animal breeding unit of National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt. They were housed under appropriate conditions of controlled humidity, temperature and light. The study was conducted accordance with the guidelines set by the European Economic Community (EEC) regulations (Revised Directive 86/609/EEC) and approved by the Ethical Committee at NCRRT. All animals were housed collectively (6 animals in each polyethylene cages $30 \times 40 \times 40 \text{ cm} - W \times L \times H$).

2.2. Micro wave system

Microwave device was designed and constructed locally in the Radiation Physics Department; NCRRT; to study the biological effect between 0.01 and 20 GHz. The microwave exposure system consisted of the following main parts:

- The microwave generator (HP 83712B) with frequencies range between 0.01 and 20.0 GHz.
- HP 8592L Spectrum analyzer that cover range from 9 KHz to 22 GHz.
- Two horns antennas, one working as a transmitter and the other as a receiver.

The animals were exposed to microwave 950 MHz at SAR (specific absorption rate) = 0.95 W kg^{-1} (watts per kilogram) for eight weeks (1 h daily during the light period).

2.3. Preparation of kombucha tea

The kombucha was obtained from microbiology lab of the NCRRT where the author is employed. It was grown and maintained in black tea medium (Sai Ram et al., 2000).

2.4. Experimental design

1) **Control group:** Animals were administered with black tea by means of stomach tube throughout the experimental period. 2) **KT administered group:** Animals were treated with kombucha tea (0.1 ml/100 g b.w.) daily and orally by means of stomach tube for nine weeks. 3) **EMF exposed group:** Animals were exposed to 950 MHz for 1 h daily for eight weeks together with black tea administered orally. 4) **KT + EMF treated group:** Animals' administered with KT for one week, then exposed to

Table 1 – The minerals level of Brain subjected to electromagnetic radiation, and/or kombucha tea.

		Brain			
		Fe ($\mu\text{g/g}$ fresh tissue)	Zn ($\mu\text{g/g}$ fresh tissue)	Cu ($\mu\text{g/g}$ fresh tissue)	Cu/Zn ratio ($\mu\text{g/g}$ fresh tissue)
X \pm SD	Control group	28.02 \pm 3.874	21.85 \pm 1.197	2.370 \pm 0.109	0.109 \pm 0.007
	EMF group	36.31 \pm 3.688 ^(a)	17.82 \pm 1.137 ^(a)	3.021 \pm 0.17 ^(a)	0.170 \pm 0.017 ^(a)
	KT group	27.70 \pm 6.434 ^(b)	21.78 \pm 1.295 ^(b)	2.407 \pm 0.22 ^(b)	0.111 \pm 0.007 ^(b)
	KT + EMF group	34.33 \pm 5.577	23.17 \pm 3.966 ^(b)	2.539 \pm 0.15 ^(b)	0.113 \pm 0.022 ^(b)

Data were expressed as Mean \pm SD, Differences were considered significant at $p < 0.05$, ^(a)Significantly different from the control group. ^(b)Significantly different from the EMF group. EMF group: the animals were kept under influence of an electromagnetic field, KT group: treated with kombucha tea KT + EMF group: Treated with kombucha tea, and under the influence of electromagnetic radiation.

the EMF (950 MHz) concurrently with KT administration up to nine weeks. Control and KT groups were kept under the same conditions without being exposed to the electromagnetic wave.

2.5. Experimental parameters

Animals exposed to 950 MHz EMF for eight weeks, after the end of the experimental period, each group of animals were sacrificed 24 h post EMF last exposure day. The animals were sacrificed by decapitation; brain, spleen and intestine were removed and stored at -80°C in glass containers until needed for analysis. To determine the concentrations of iron, copper and zinc, tissue samples were washed thoroughly with doubly distilled water; prepared by ELGA Ultra Pure Water Station (England). The weighed samples were digested by adding mixture of concentrated nitric acid and hydrogen peroxide (H_2O_2) in 5:1 ratio respectively (IAEA, 1980, p. 379). Samples digestion was carried out with acids at elevated temperature and pressure by using Microwave Sample Preparation; Labstation MLS-1200 (MEGA, Italy). Samples diluted with doubly distilled water to appropriate concentration level (Kingstone & Jassie, 1988, 263 pp.). Iron, zinc and copper were estimated in brain, spleen and intestine. Concentration of elements in tissues was calculated by using calibration curve prepared from their stock solution (1 mg). The concentration of elements per grams wet tissues could be determined by the following equation (Gregus & Klaassen, 1986).

$$C1 = C2/W \times D$$

C1; concentration of the element per gram wet tissue ($\mu\text{g/g}$)
C2; concentration of the sample solution ($\mu\text{g/ml}$)

W; sample weight

D; dilution factor of the sample.

2.6. Statistical analysis

The data were expressed as mean \pm standard deviation (SD) and were analyzed by one way analysis of variances (ANOVA) followed by Tukey's multiple comparison test. Statistical analysis was performed by using Graph-Pad software, San Diego, CA, USA. Differences were considered statistically significant when $P < 0.05$.

3. Results

Non-significant increase was observed in all parameters upon kombucha administration to rats as compared to control group.

Concerning the concentration levels of Fe, Zn, Cu and Cu/Zn ratio in brain tissues; EMF administration imposes deleterious effect as evidenced by significant increase in Fe, Cu concentration as well as Cu/Zn ratio accompanied with a significant depletion in Zn concentration. Concomitant administration of kombucha with EMF caused an improvement of all these elements concentration (Table 1).

As shown in Table 2 exposure to 950 MHz EMF for eight weeks lead to a marked increase in the level of spleen Fe, Cu, and Cu/Zn ratio accompanied with significant decrease in spleen Zn concentration. Administration of kombucha tea during exposure to electromagnetic wave maintained the level of these metals to their normal levels.

Table 2 – The minerals level of Spleen subjected to electromagnetic radiation, and/or kombucha tea.

		Spleen			
		Fe ($\mu\text{g/g}$ fresh tissue)	Zn ($\mu\text{g/g}$ fresh tissue)	Cu ($\mu\text{g/g}$ fresh tissue)	Cu/Zn ratio ($\mu\text{g/g}$ fresh tissue)
X \pm S.D	Control group	258.4 \pm 7.49	34.695 \pm 3.45	1.626 \pm 0.281	0.049 \pm 0.007
	EMF group	327.7 \pm 53.69 ^(a)	26.58 \pm 2.368 ^(a)	1.834 \pm 0.076	0.067 \pm 0.008 ^(a)
	KT group	264.0 \pm 42.04 ^(b)	36.24 \pm 3.5 ^(b)	1.416 \pm 0.023 ^(b)	0.0412 \pm 0.0016 ^(b)
	KT + EMF group	314.9 \pm 27.94	33.08 \pm 1.38 ^(b)	1.753 \pm 0.310	0.055 \pm 0.0096 ^(b)

Data were expressed as Mean \pm SD, Differences were considered significant at $p < 0.05$, ^(a)Significantly different from the control group. ^(b)Significantly different from the EMF group. EMF group: the animals were kept under influence of an electromagnetic field, KT group: treated with kombucha tea KT + EMF group: Treated with kombucha tea, and under the influence of electromagnetic radiation.

Table 3 – The minerals level of Intestine subjected to electromagnetic radiation, and/or kombucha tea.

		Intestine			
		Fe ($\mu\text{g/g}$ fresh tissue)	Zn ($\mu\text{g/g}$ fresh tissue)	Cu ($\mu\text{g/g}$ fresh tissue)	Cu/Zn ratio ($\mu\text{g/g}$ fresh tissue)
X \pm SD	Control group	62.66 \pm 10.33	52.65 \pm 8.92	1.82 \pm 0.141	0.0395 \pm 0.0048
	EMF group	99.59 \pm 3.80 ^(a)	35.49 \pm 4.66 ^(a,b)	2.394 \pm 0.121 ^(a)	0.067 \pm 0.0049 ^(a)
	KT group	65.89 \pm 4.72 ^(b)	62.46 \pm 10.87	2.013 \pm 0.170 ^(b)	0.0347 \pm 0.011 ^(b)
	KT + EMF group	75.21 \pm 9.49 ^(a,b)	44.44 \pm 8.05 ^(b)	2.343 \pm 0.031 ^(a)	0.0547 \pm 0.011 ^(a)

Data were expressed as Mean \pm SD, Differences were considered significant at $p < 0.05$, ^(a)Significantly different from the control group. ^(b)Significantly different from the EMF group. EMF group: the animals were kept under influence of an electromagnetic field, KT group: treated with kombucha tea KT + EMF group: Treated with kombucha tea, and under the influence of electromagnetic radiation.

Exposure of rats to 950 MHz EMF for eight weeks caused a significant increase in Fe, Cu and Cu/Zn ratio accompanied with a decrease in level of intestine Zn. Administration of KT concurrently with EMF exposure resulted in significant increases in Fe, Cu levels and Cu/Zn ratio and a non-significant decrease as shown in intestine Zn level comparing to the control levels (Table 3).

4. Discussion

The biological effects of electromagnetic radiation including that emitted from mobile phones have been linked to reactive oxygen species lead to increased free radical production and lipid peroxidation in some tissues (Gharib, 2011; Guney et al., 2007).

Researches show that iron, copper, and zinc are competitive. Copper is necessary for the conversion of iron to hemoglobin, but if there is excess zinc, less iron will be available for conversion, because zinc is competing. This may result in a secondary deficiency of iron, which can manifest itself as iron deficiency anemia. All may be due simply to excess zinc (Eaton & Qian, 2002; Welch, Davis, Van Eden, & Aust, 2002).

In addition, copper and zinc are believed to play an important role in carcinogenesis. Several studies have shown elevation of copper and decrease of zinc in various malignancies (Gupta, Singh, & Shukla, 2005). Copper is an essential element that contributes to important intracellular metabolic events and copper ion levels are elevated in a number of malignancies (Han et al., 2003). Also, the zinc and copper levels of animals were drastically changed by irradiation (Dede et al., 2003). Our data are consistent with these reports. Lower zinc levels in all tested organs may be evidence of their damages due to electromagnetic waves.

In the present study, Fe and Cu showed a significant increase in their concentration due to electromagnetic radiation exposure. This may be due to the oxidative stress resulted from EMF exposure (Gharib, 2011; Guney et al., 2007). This increase lead to a cellular depletion of enzyme activities accompanied with a generation of reactive species (Chen, Ding, Castranova, & Shi, 2001). On the other hand, zinc concentrations in all organs tissue were higher in the controls than in EMF subjected group, accompanied with an increase in Cu/Zn ratio. These differences between control and EMF exposure are interesting, since zinc has an activating effect on the antioxidant system and its deficiency could be

accompanied with the increase in lipid peroxidation (Guo, Chen, Yeh, Hsiung, & Wang, 2011; Ozturk et al., 2001; Powell, 2000). It should be noted that the lowest zinc levels in EMF animals group is a result of exposure to an EM field (Ozturk et al., 2001). Moreover, large concentration of iron might decrease zinc absorption causing zinc deficiency (Whittaker, 1998).

A mutual antagonism related to the absorption of zinc and copper has been reported (Zowczak, Iskra, Torlinski, & Cofta, 2001). Some authors have reported a strong correlation between the stage of various malignancies and elevated serum copper levels and copper/zinc ratio on one hand and decreased serum zinc levels on the other (Han et al., 2003). In this study, a significant increase in the copper/zinc ratio was observed in brain, spleen and intestine as compared to the controls. This is accompanied by a significant decrease in zinc level in all tested organs due to the electromagnetic radiation exposure.

Investigation revealed that kombucha contains amino acids which are constituents of proteins, produced antioxidant enzymes and glutathione; a powerful antioxidant, which provides protection from environmental pollutants (Blanc, 1996; Steiger & Steinegger, 1957). Kombucha cultures contain two groups of symbiotic microbes: Xylinum and yeasts. Xylinum generates bacterial cellulose from ethanol produced by the yeast; bacterial cellulose is an efficient adsorbing agent that will adhere to metals ions and other substances thereby facilitating their removal (Wang & Zhang, 2011). The present results indicated that administration of kombucha to rats exposed to the electromagnetic waves showed amelioration in the studied trace elements homeostasis which may be due to the adsorption effects of bacterial cellulose produced by the kombucha culture. On the other hand, reduced glutathione helps in the reduction of heavy metal ions by accepting electrons (Galhardi et al., 2004).

In conclusion the administration of kombucha to rats exposed to EMW could achieve trace elements homeostasis in different organs.

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